CHARGE SEPARATION OF PROTEINS COMPLEXED WITH SODIUM DODECYL SULFATE BY ACID GEL ELECTROPHORESIS IN THE PRESENCE OF CETYLTRIMETHYLAMMONIUM BROMIDE

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Summary

Globular proteins, casein, and membrane proteins which were reacted with sodium dodecyl sulfate were studied by acid urea gel electrophoresis. The sodium dodecyl sulfate bound tightly to the proteins, producing a more acidic charge which prevented migration into the gel. When cetyltrimethylammonium bromide was added to the sodium dodecyl sulfate-protein complexes, the sodium dodecyl sulfate apparently reacted with cetyltrimethylammonium bromide and dissociated so that the proteins migrated in acid gel in a normal manner as compared to the proteins without any added detergent. The sodium dodecyl sulfate-cetyltrimethylammonium bromide complex could be removed from the proteins by centrifugation. Thus, cetyltrimethylammonium bromide used in conjunction with acid gel electrophoresis allows direct comparison by charge of proteins fractionated in the presence of sodium dodecyl sulfate with the starting mixture of proteins not exposed to detergent. The reaction of cetyltrimethylammonium bromide with sodium dodecyl sulfate in acidic urea also provides a simple convenient method of removal of sodium dodecyl sulfate from proteins.

Introduction

Polyacrylamide gel electrophoresis in various media (acidic, basic, sodium dodecyl sulfate (SDS)) is an excellent method of testing the homogeneity of

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then stirred at 4°C for about 5 h and allowed to stand at 4°C overnight. The protein was dialyzed extensively against deionized water at 4°C and was then lyophilized and stored at 4°C.

Determination of SDS bound to protein

The amount of protein-bound SDS was estimated by the methylene blue method of Weil and Stirton [10] with sodium dodecyl sulfate as a standard.

Polyacrylamide gel electrophoresis

Proteins were examined by polyacrylamide gel electrophoresis at acid pH [11], on an EC-Vertical Slab Gel Apparatus. The stock buffer was made by diluting 86 ml of glacial acetic acid and 25 ml of 90% formic acid to 1 l. The gels were prepared from a solution containing 10% Cyanogum 41, 4.5 M urea, and 0.2% thiourea. The gel was polymerized by the addition of 30% hydrogen peroxide to give a final concentration of 0.5%. The solution was poured quickly into the cell and immediately a Teflon slot former was inserted since polymerization was nearly complete within 5 min. For electrode buffer, 7% acetic acid was used.

The sample (2.5 mg) was dissolved in 90 μl solution of 0.5% Na_2CO_3 and 8 M urea and reduced by the addition of 20 μl of 2-mercaptoethanol. It was left standing at room temperature for about 1.5 h. In order to lower the pH of sample solution to 3, 180 μl of sucrose solvent system (20% sucrose and 10 M urea dissolved in stock buffer) and 10 μl of 2-mercaptoethanol were added. The cetyltrimethylammonium bromide solvent system consisted of 5% CTAB and sucrose solvent. The amount of CTAB required to neutralize the SDS in the samples was calculated, and the volume of sucrose solvent was adjusted accordingly for each sample. The sample solution was then heated at 37°C for 45 min to insure SDS and CTAB would react, and 10 μl of tracking dye (1 mg methyl red/1 ml ethanol) were added before electrophoresis.

The gels were run for 7 h at 90 mA and maintained at 14°C. After the run, the gels were stained for protein with 0.03% Coomassie blue in 10% trichloroacetic acid/10% methanol/7% acetic acid and destained by washing several times with 10% methanol/7% acetic acid.

Results and Discussion

Sodium dodecyl sulfate (SDS) has been used to effectively dissociate proteins from membranes. However, the detergent often remains tightly bound to the protein even after exhaustive dialysis. Three standard proteins as well as two fractions of the fat globule membrane were reacted with SDS as described in Materials and Methods; after exhaustive dialysis the proteins were lyophilized and analyzed for SDS content. The results are shown in Table I. The percent SDS bound ranged from 20 to 30% as given by the methylene blue test. In two cases this was checked by weight percent based on recovery. Thus, all of the proteins contained tightly bound SDS even after exhaustive dialysis. When these proteins were subjected to acid gel electrophoresis without further treatment, the proteins remained for the most part in the sample slots or migrated into the anodal electrode buffer. However, when treated with cetyltrimethylam-

TABLE I

DETERMINATION OF SDS BOUND TO PROTEINS

Sample *	After complex formation		After removal of SDS by CTAB
	% SDS (methylene blue test)	% SDS (by recovered weight)	% SDS (methylene blue test)
β-Lactoglobulin A	20.5	20.6	1.81
α-Casein	20.4	20.6	—
α-Lactalbumin	29.4	—	2.48
Bovine associated mucoprotein	30.0	—	—
Soluble fraction	28.7	—	0.84

* SDS-protein complexes prepared as described in text.

monium bromide (CTAB) as described in Materials and Methods, the bands obtained were sharp and identical with those of proteins which were not reacted with SDS.

Fig. 1 shows the electrophoretic patterns of native β-lactoglobulin A in the absence and in the presence of added CTAB. Fig. 1a (no detergent) shows two major bands; Fig. 1b shows that CTAB alone did not interfere with the migration of the β-lactoglobulin. An anomalous fast moving band was observed in Fig. 1b. Blank runs were performed on samples containing no protein but with different molar ratios of SDS : CTAB. All samples which contained a ratio of more than 1 did not show any band at the front of the gel. A front band became observable only when there was excess CTAB in the samples. This

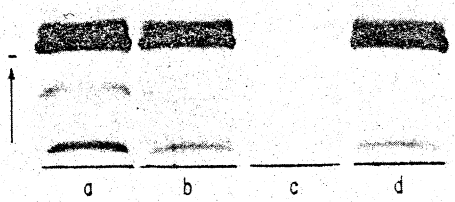

Fig. 1. Polyacrylamide gel electrophoresis of β-lactoglobulin A in acetic acid/formic acid buffer, pH 3.0, 10% acrylamide and 4.5 M urea: (a) native protein without detergents, (b) native protein in the presence of CTAB, (c) protein complexed with SDS prepared as described in text, and (d) SDS-protein complex in the presence of CTAB.

experiment indicated that CTAB formed a front which was stained easily with Coomassie blue. When CTAB was added to SDS, birefringence was observed; these insoluble particles did not change the electrophoretic patterns since they apparently remained in the slots of the gel. Fig. 1c shows that SDS-protein complex did not migrate in the acid gel because of the high net negative charge of the complex. But when CTAB was added to SDS-protein complex, birefringence was observed in the samples (Fig. 1d), and the protein bands migrated in a normal fashion as the ones shown in Figs. 1a and 1b. This is probably because CTAB has reacted with SDS to form a neutral compound which causes birefringence and does not migrate into the gel. Alternatively, the SDS-protein-CTAB complex could comigrate with the native protein.

In order to determine if CTAB actually removes SDS from SDS-protein complexes, scaled-up (4 ml) SDS-CTAB gel samples were centrifuged at 100 000 $\times g$ for 1 h at 20°C. A detergent pellicle was removed by aspiration and the solvent layer was dialyzed and lyophilized. Three proteins were analyzed for SDS content as shown in the last column in Table I. The values demonstrate that 91 to 95% of the SDS has been removed in one step. The results present a good evidence that SDS is removed from proteins by CTAB in the presence of acidic urea. Extension of the method to other pH values could conceivably be accomplished. Preliminary experiments at higher pH values showed that several variables need to be carefully controlled; these include the molarity of urea necessary to solubilize the proteins, the ratio of SDS to CTAB, and the nature of the protein studied. The method appears to have its widest application in acidic urea.

Figs. 2 and 3 illustrate the electrophoretic patterns of whole α-casein and

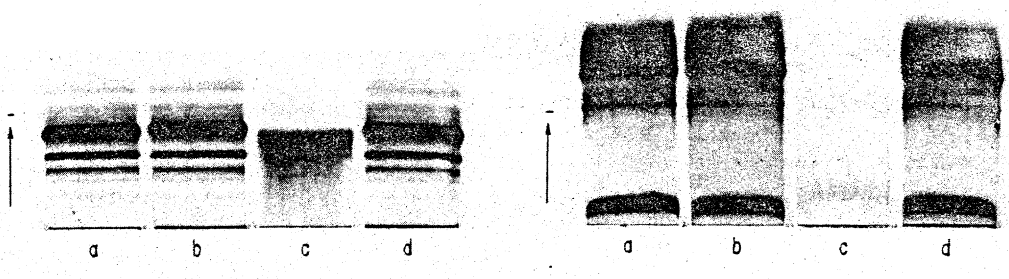

Fig. 2. Polyacrylamide gel electrophoresis of whole α-casein as described in Fig. 1.

Fig. 3. Polyacrylamide gel electrophoresis of crude α-lactalbumin as described in Fig. 1.

α-lactalbumin, respectively. Patterns a and b show the native proteins unreacted with SDS in the absence and in the presence of added CTAB. Again, there is no effect by CTAB on the migration of the proteins. When the proteins reacted with SDS, these complexes showed little or no mobility (Figs. 2c and 3c). When CTAB was added to these complexes (pattern d), the proteins exhibited their normal electrophoretic mobilities. In Figs. 1—3 the proteins without the SDS and the SDS-protein complexes are compared (pattern b vs. pattern d). There are no missing bands or new bands in the gels.

The electrophoretic pattern of bovine associated mucoprotein, which is derived from the fat globule membrane of milk, is shown in Fig. 4. The use of the detergents was the same in Fig. 4 as in Figs. 1—3. Figs. 4a and 4b demonstrate that CTAB did not interfere with the migration of bovine associated mucoprotein, while Figs. 4c and 4d show that the CTAB will dissociate SDS from these membrane proteins as well as from globular proteins and casein.

When bovine associated mucoprotein is compared to the soluble protein fraction of fat globule membrane [3] as shown in pattern a of Figs. 4 and 5, it is evident that they are not quite similar, although they appear nearly identical on the basis of SDS-polyacrylamide gel electrophoresis. This illustrates the importance of electrophoresis at acid pH. When the whole soluble fraction of the fat globule membrane protein was reacted with SDS, fractions were recovered from gel column chromatography. The presence of SDS in proteins hindered their migration in the acid gel (Fig. 5c); CTAB removed the undesired effect of SDS on proteins as exemplified by crude glycoprotein B (Fig. 5d).

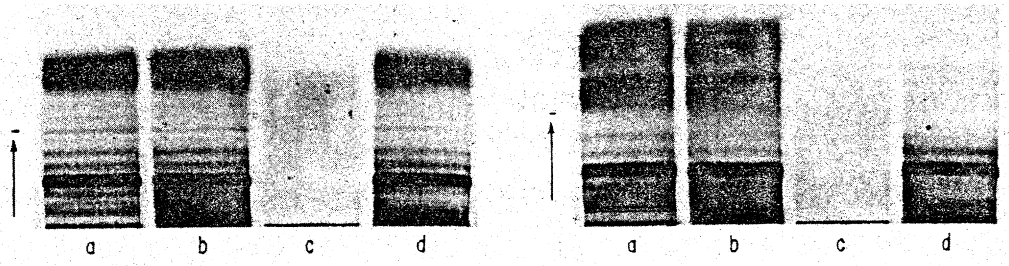

Fig. 4. Polyacrylamide gel electrophoresis of bovine associated mucoprotein as described in Fig. 1.

Fig. 5. Polyacrylamide gel electrophoresis of: (a) soluble protein fraction of milk fat globule membrane, (b) CTAB added to soluble protein fraction, (c) the major protein of the soluble fraction of the fat globule membrane, obtained by SDS gel chromatography, and (d) CTAB added to the SDS-chromatographed protein shown in pattern c.

This technique allows direct comparison by charge of the protein which was fractionated by SDS gel chromatography with the starting material.

This method shows a good analytical application as well as a preparative application in the removal of sodium dodecyl sulfate from proteins with the aid of CTAB in acid urea. Methylene blue analysis for SDS showed that 20—30% of the dry weight of the above-mentioned proteins was due to bound SDS. It appeared that the dialysis failed to remove all of the SDS from the proteins. Since sodium dodecyl sulfate has been a satisfactory agent for isolating individual proteins of the milk fat globule membrane, the use of CTAB provides a convenient method for the removal of SDS from proteins on a larger scale.

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